Journal club: April 09, 2013

Genome Engineering Using CRISPR

RNA-Guided Human Genome Engineering via Cas9

Prashant Mali,[1]* Luhan Yang,[1,3]* Kevin M. Esvelt,[2] John Aach,[1] Marc Guell,[1] James E. DiCarlo,[4] Julie E. Norville,[1] George M. Church[1,2]†

SCIENCE VOL 339 15 FEBRUARY 2013

Multiplex Genome Engineering Using CRISPR/Cas Systems

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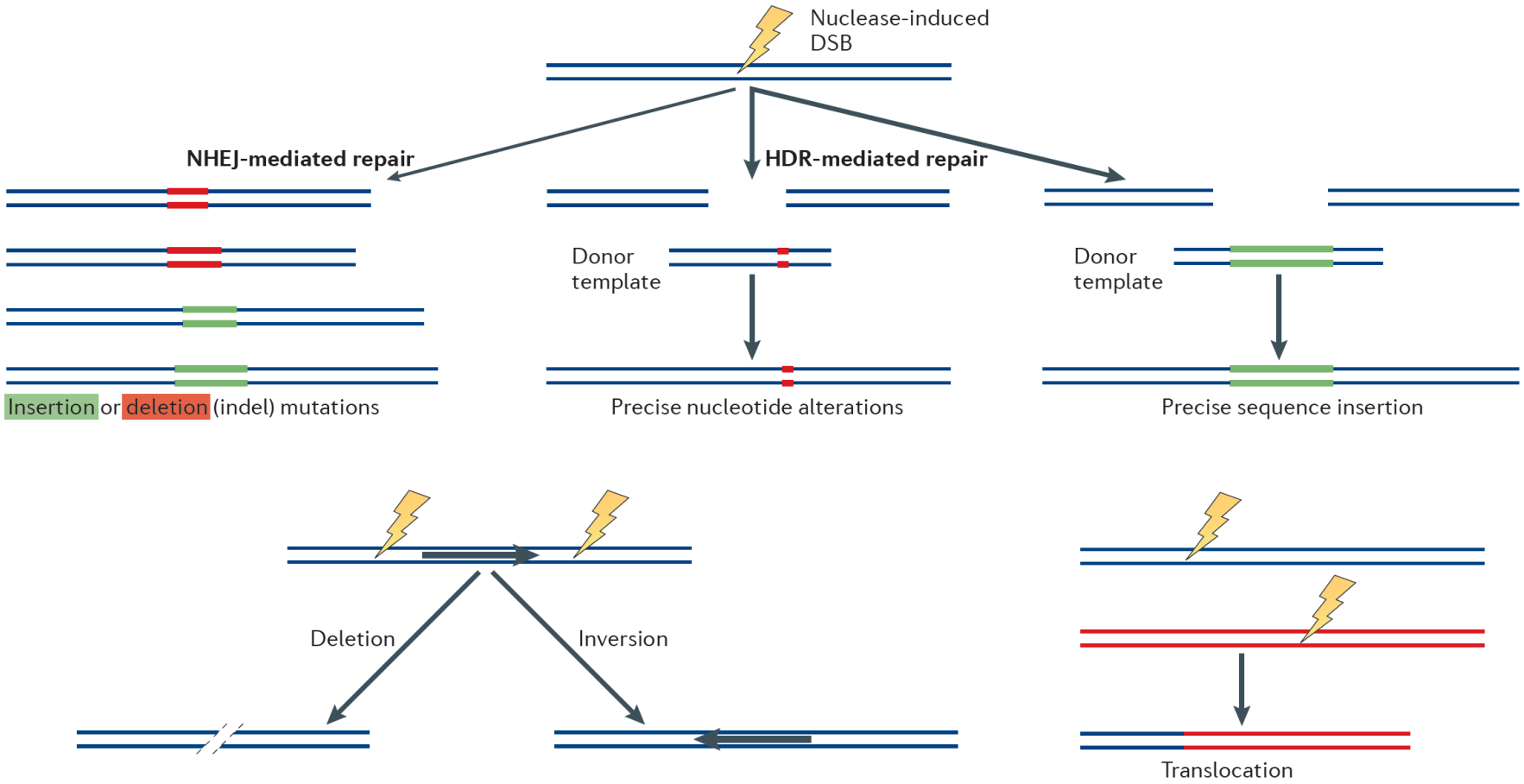
background - genome editing



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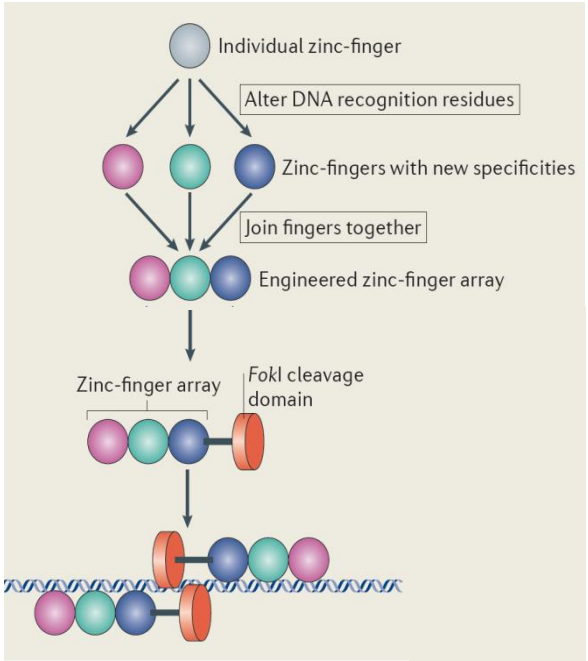
VOLUME 14 | JANUARY 2013

genome editing – how to induce DSB?

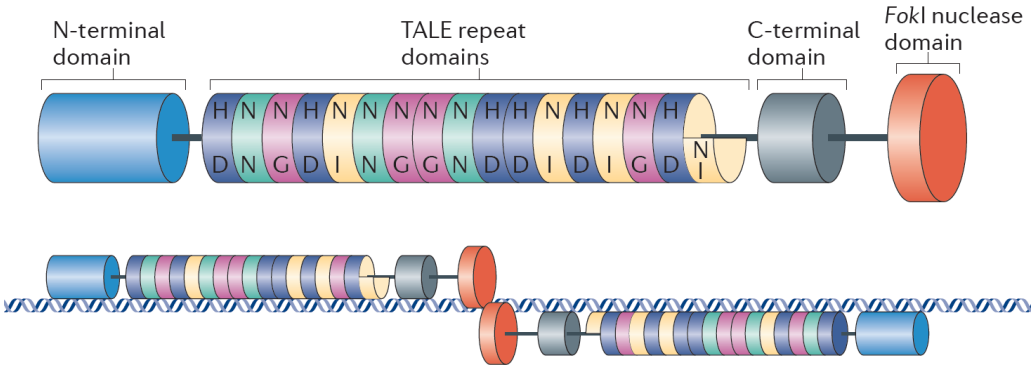
zinc-finger nucleases (ZFNs)

- Cys2His2 DNA binding-domains
- recognize approx. 3 bp
- modifying DNA binding specificity
- array-design
- fusion with nonspecific endonuclease domain



transcription activator-like effector nucleases (TALENs)

- 33-35 aa bacterial repeats
- specifically binding to 1 base of DNA
- array-design
- fusion with nonspecific endonuclease domain



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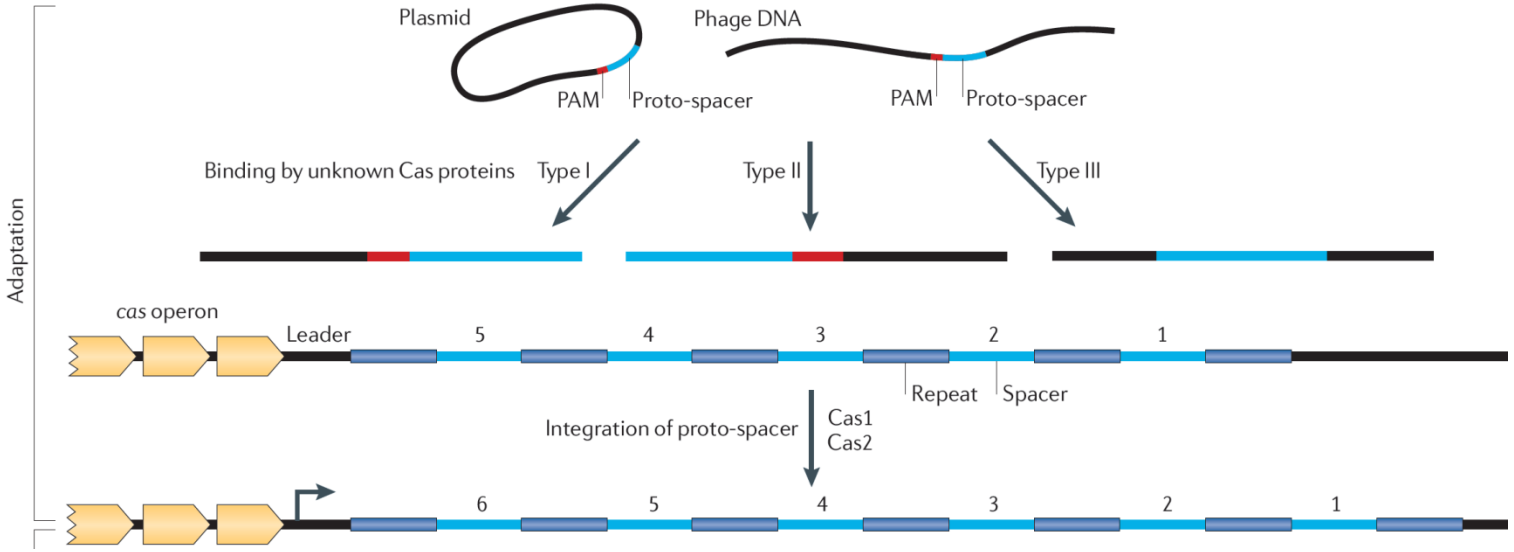
background - CRISPR

the adaptive immune system of bacteria and archaea

- **c**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats
- found in 90 % of archaea and 40 % of bacteria
- direct repeats + spacers
- cas gene (CRISPR-associated)
- proto-spacer adjacent motiv (PAM)



Evolution and classification of the CRISPR–Cas systems
Kira S. Makarova
NATURE REVIEWS | MICROBIOLOGY
VOLUME 9 | JUNE 2011 | 467

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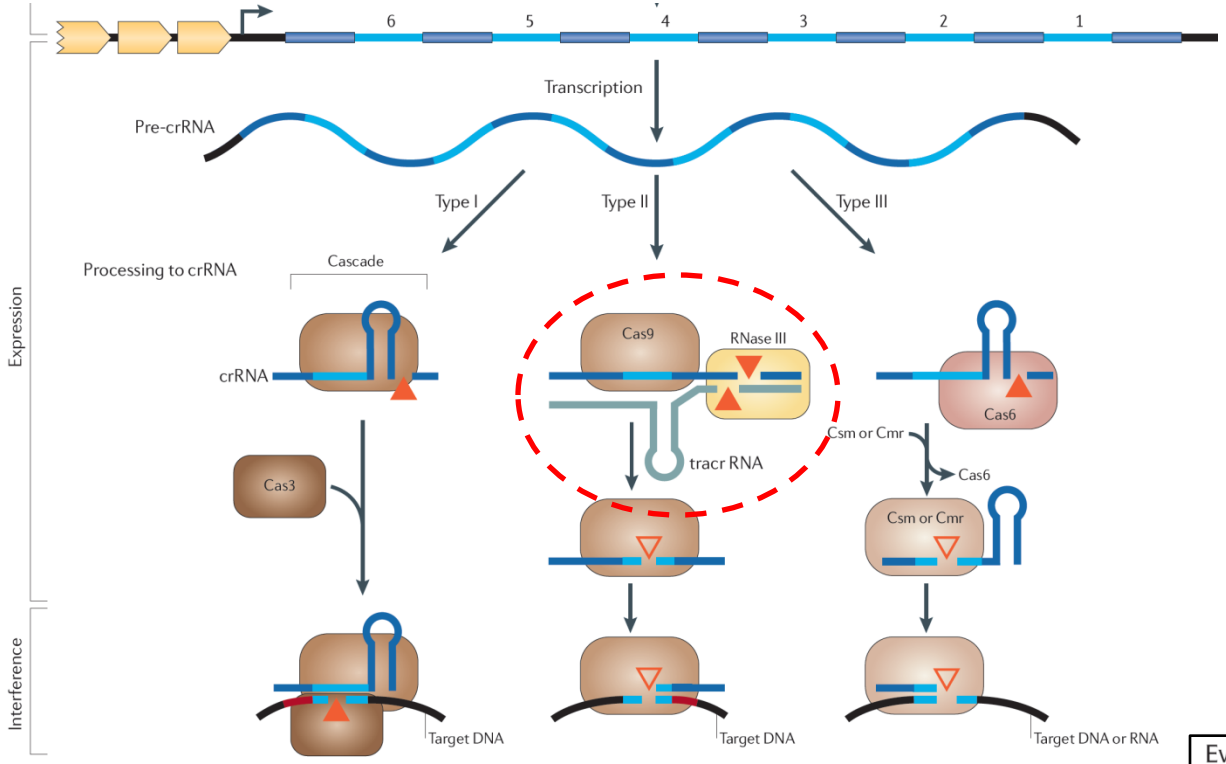
- pre-crRNA processed into crRNAs (23-47 nt)
- matches proto-spacer of invading nucleic acid
- Type II uses a trans-encoded small RNA (tracrRNA)



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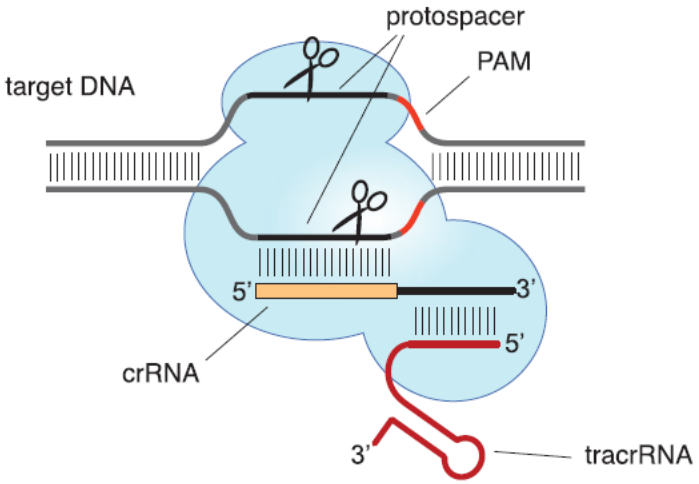
Cas9 programmed by crRNA:tracrRNA duplex



A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity
Martin Jinek *et al.*
SCIENCE VOL 337 17 AUGUST 2012

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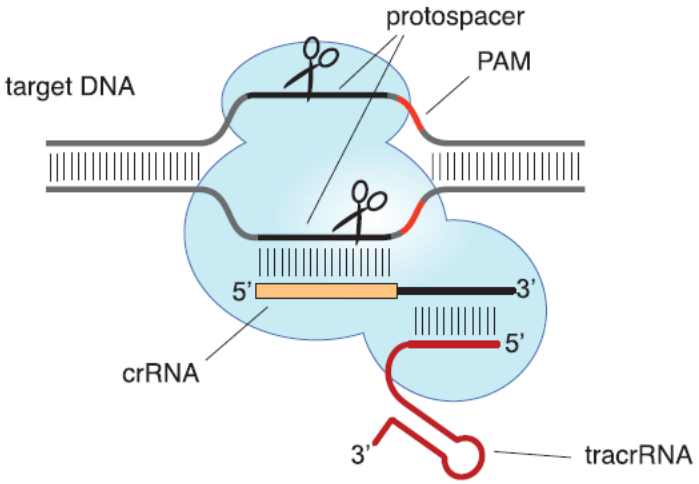
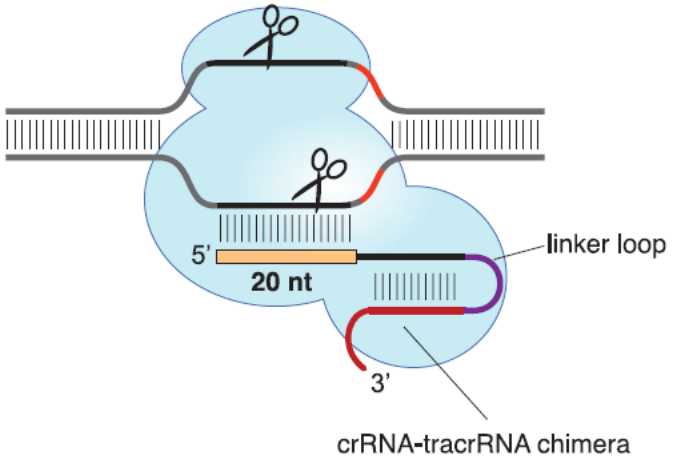


Cas9 programmed by single chimeric RNA



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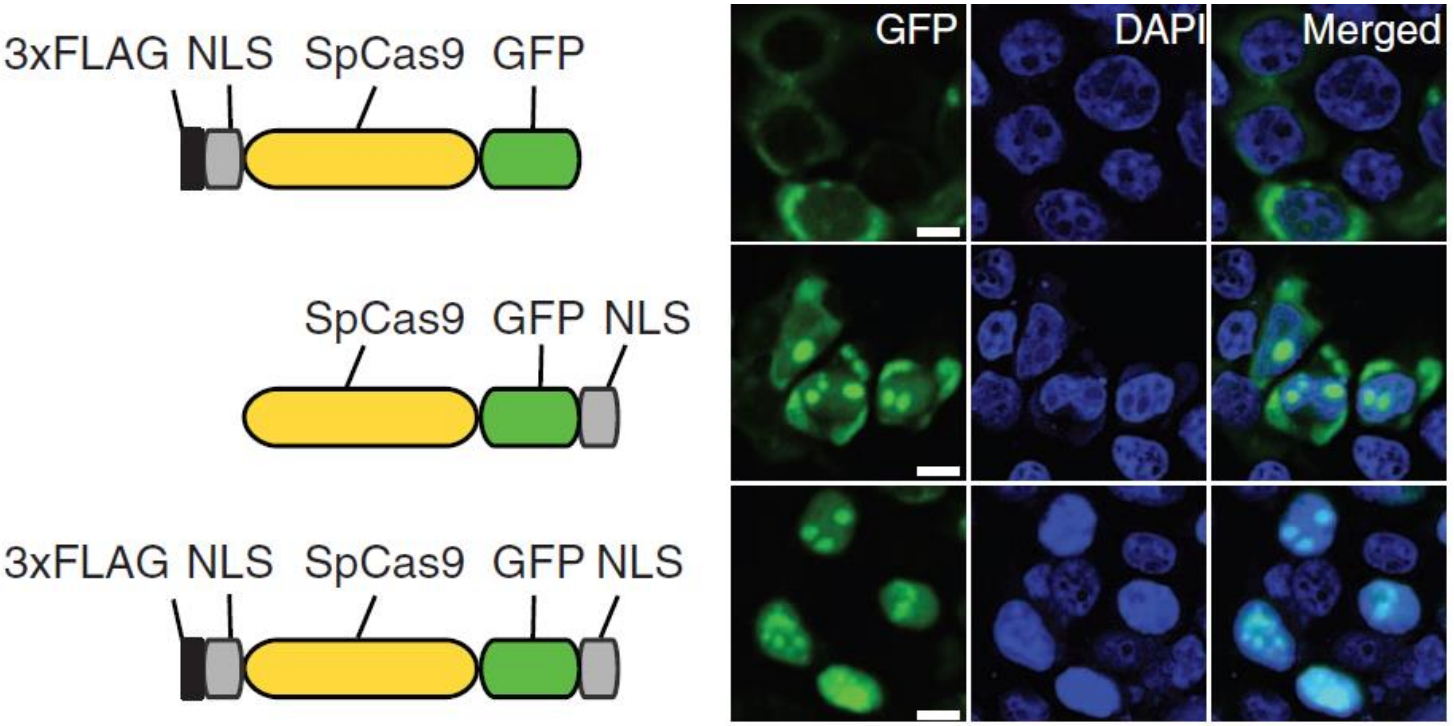
construction of the system

type II CRISPR locus from *S. pvogenes*

- codon-optimized Cas9
- introduced NLS



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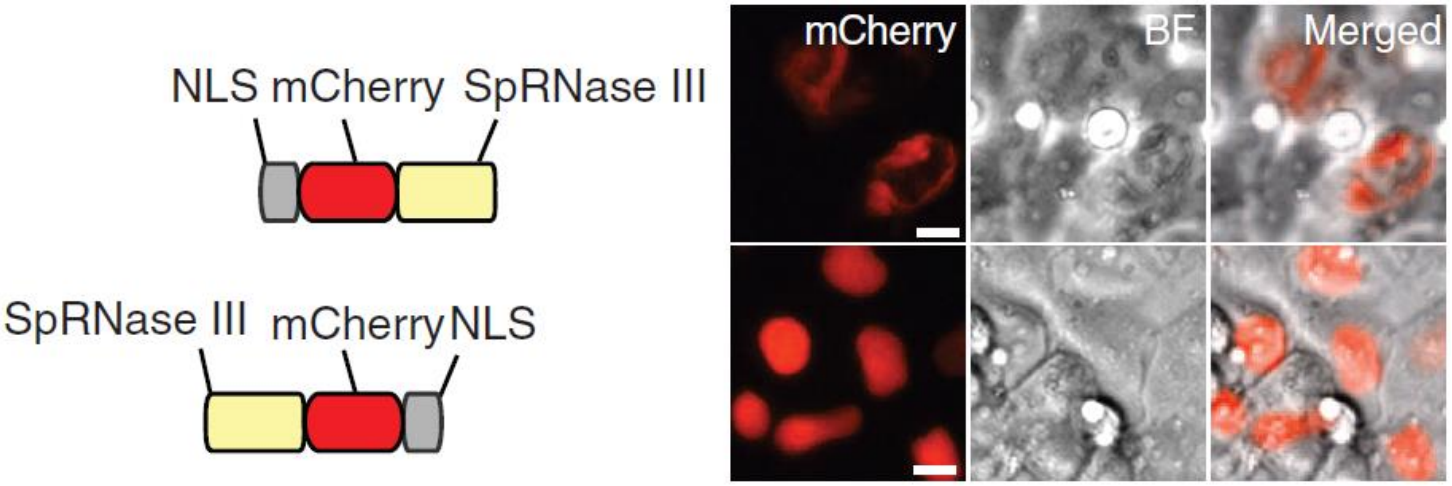

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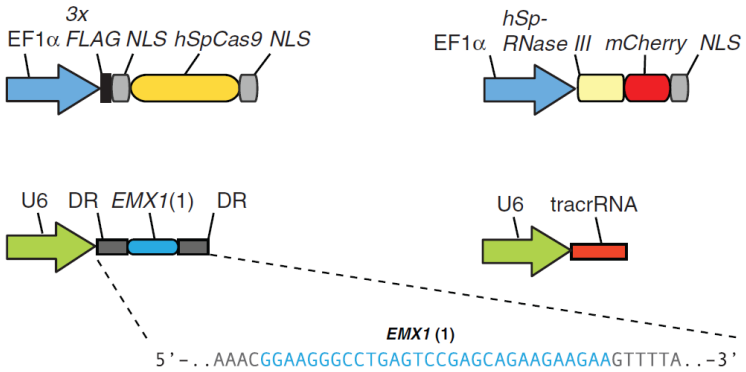
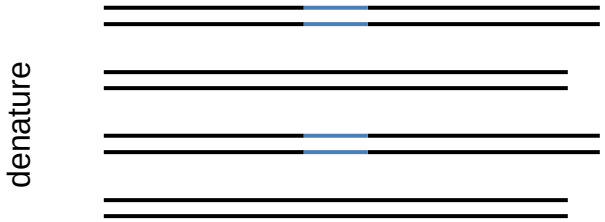
first tests (NHEJ)



- transiently express constructs in HEK293
- pooled genomic DNA
- amplified EMX locus
- **survey assay**
  - denature DNA



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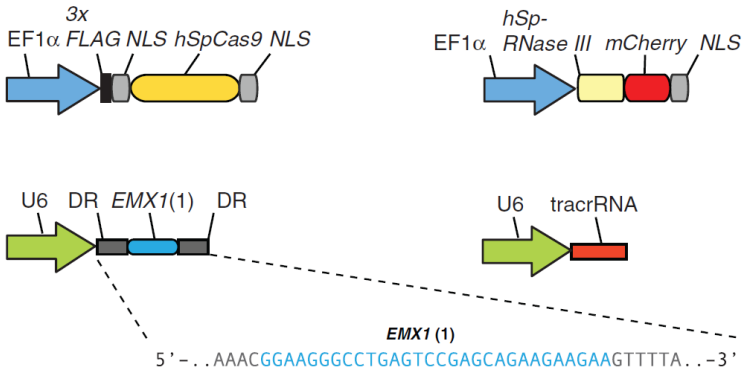
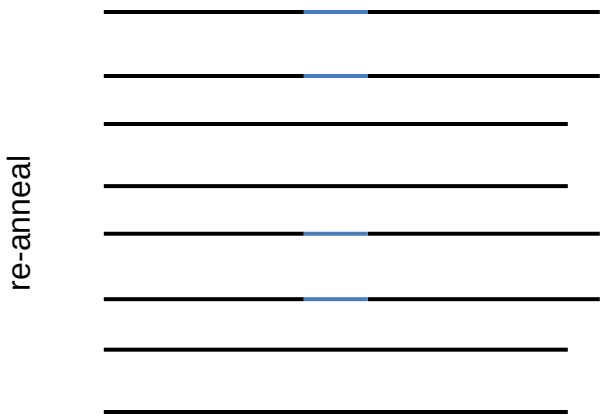


- transiently express constructs in HEK293
- pooled genomic DNA
- amplified EMX locus
- **survey assay**
  - denature and re-anneal DNA



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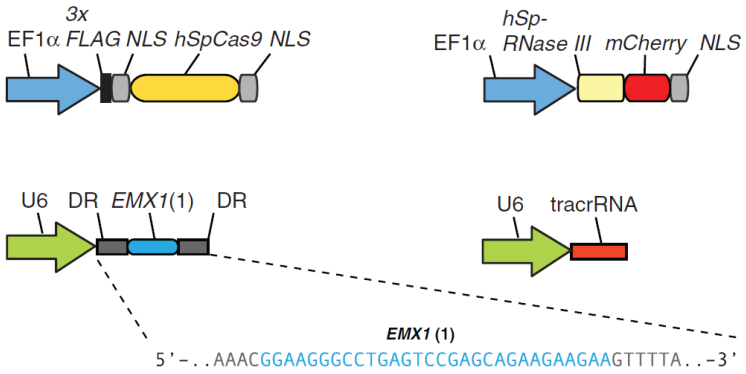
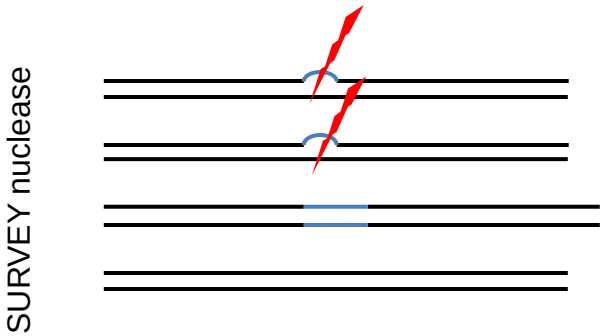


- transiently express constructs in HEK293
- pooled genomic DNA
- amplified EMX locus
- **survey assay**
  - denature and re-anneal DNA
  - heteroduplexes are cut by SURVEY nuclease



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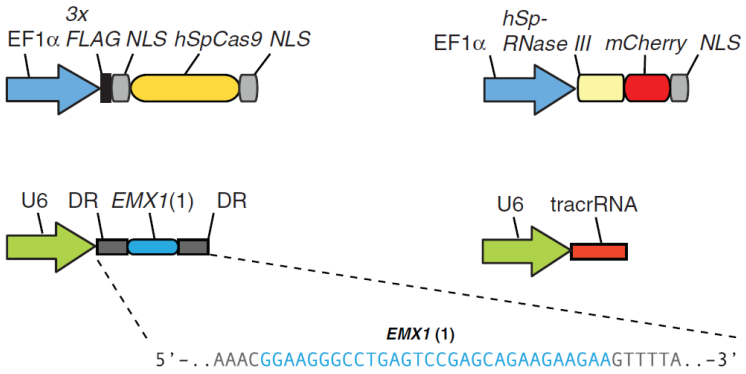
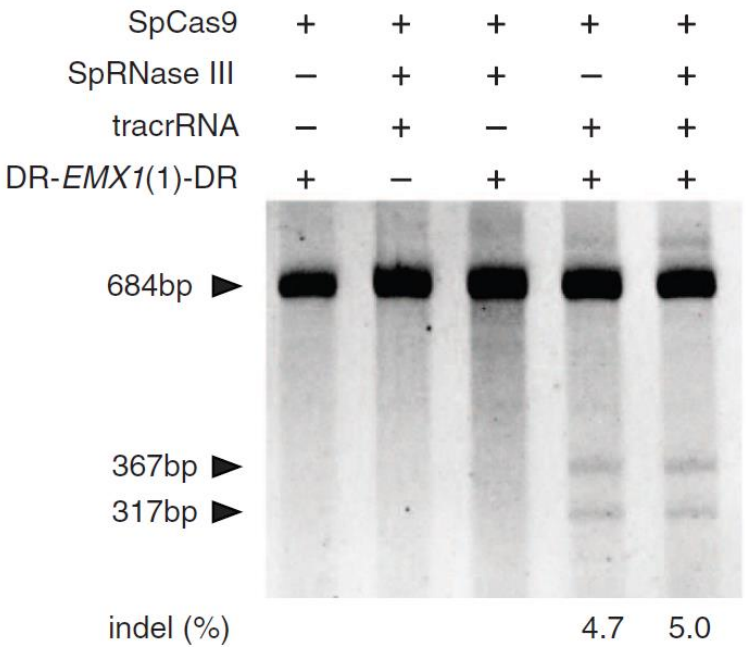


- transiently express constructs in HEK293
- pooled genomic DNA
- amplified EMX locus
- **survey assay**
  - denature and re-anneal DNA
  - heteroduplexes are cut by SURVEY nuclease
- Sanger seq



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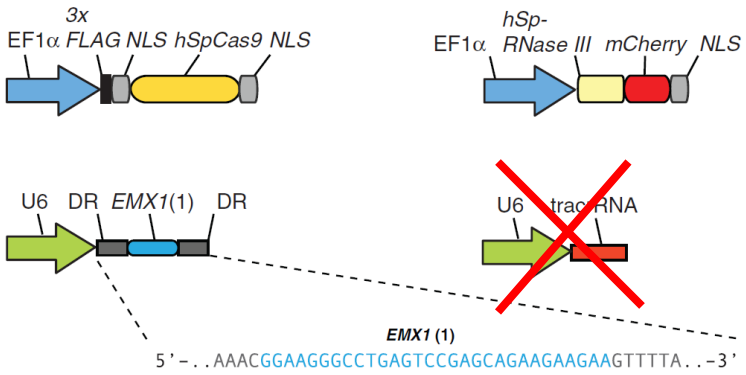

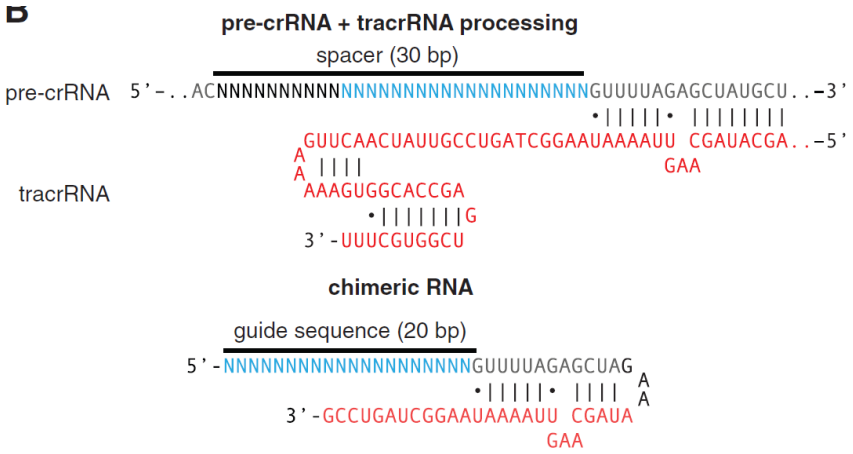

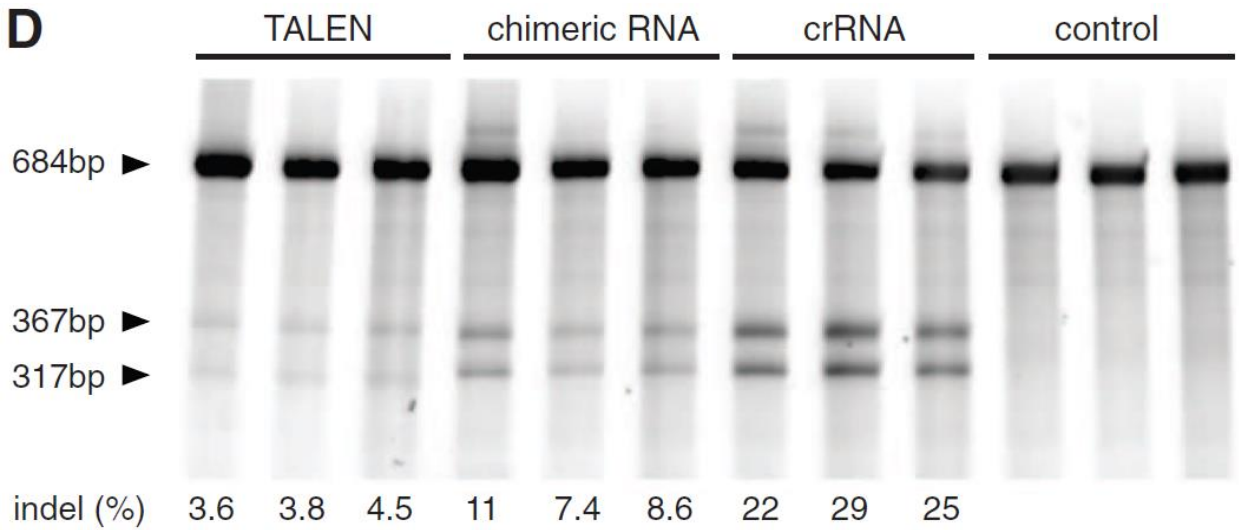

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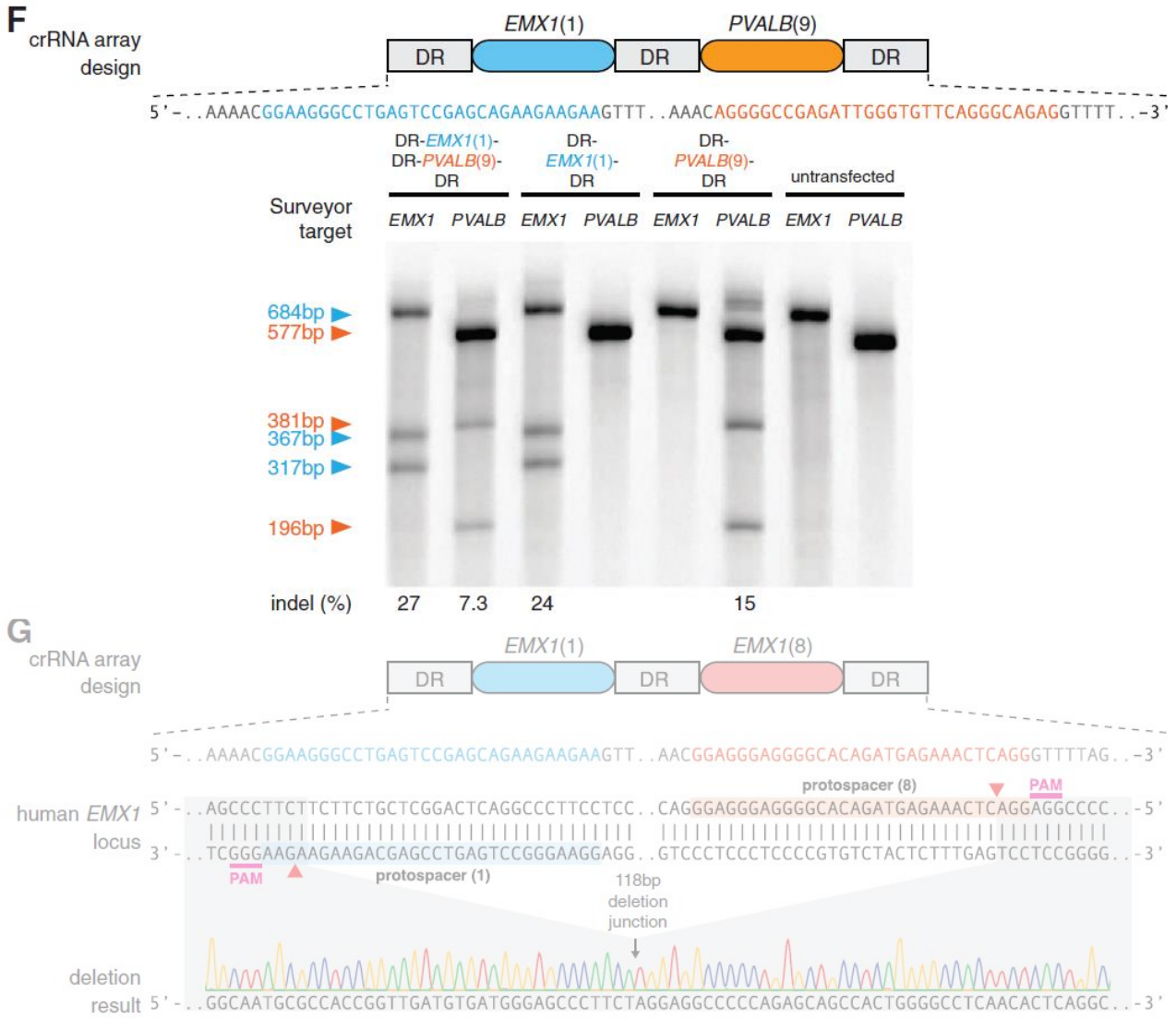
array design (NHEJ)



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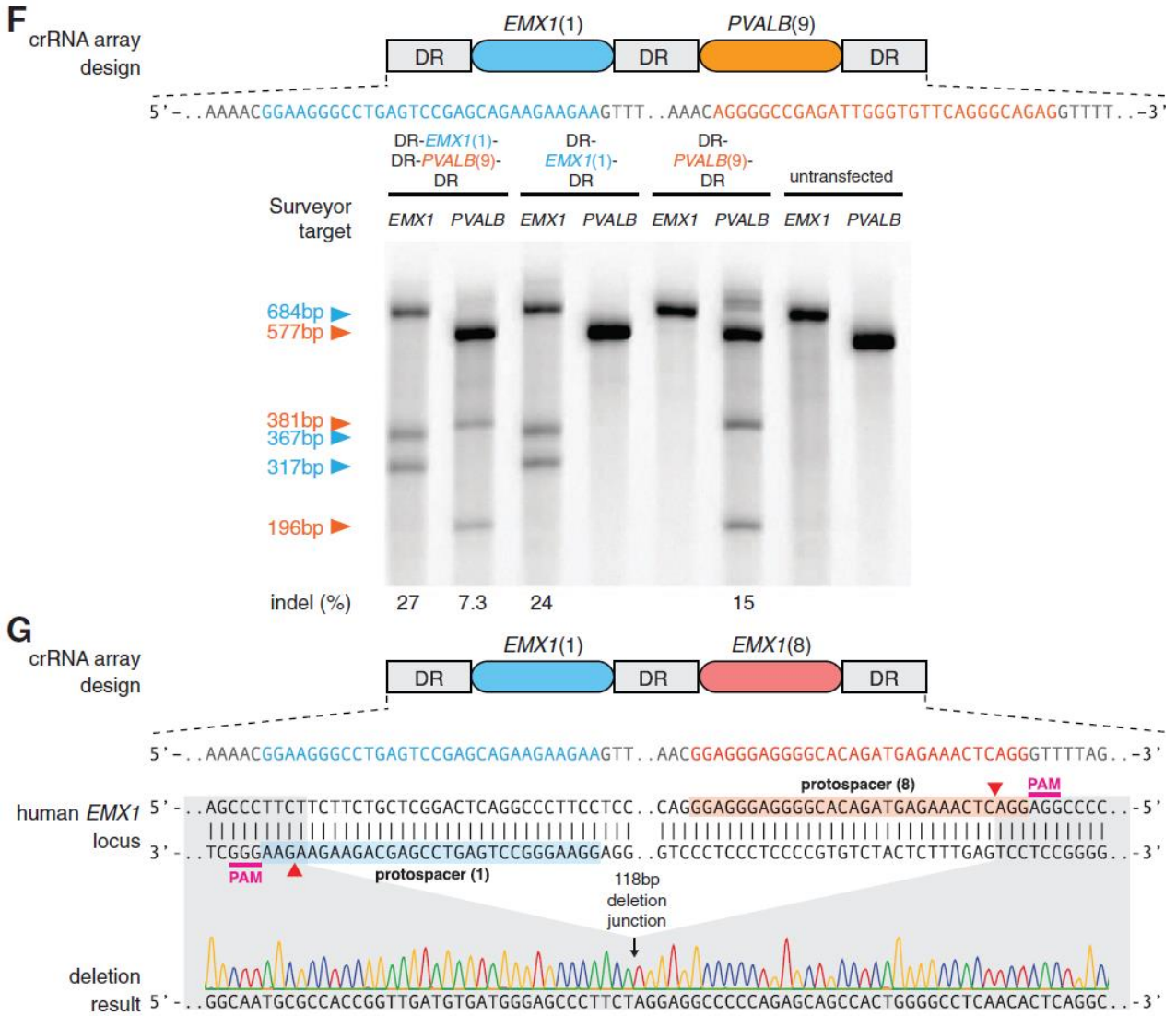

1.6 % deletion efficiency

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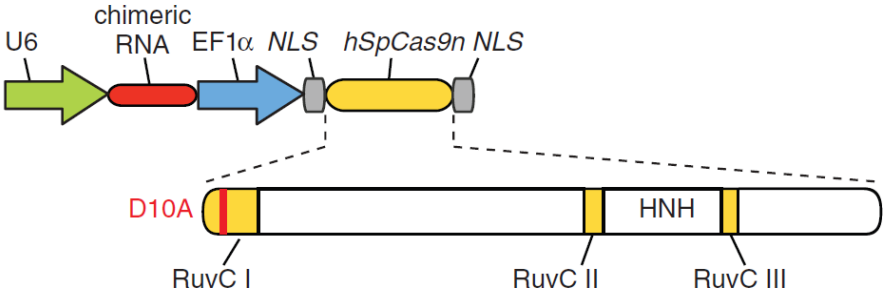
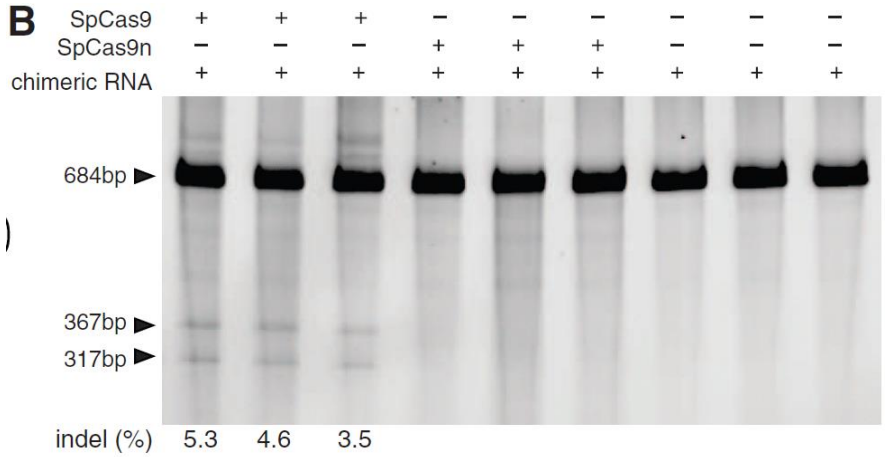
homologous recombination





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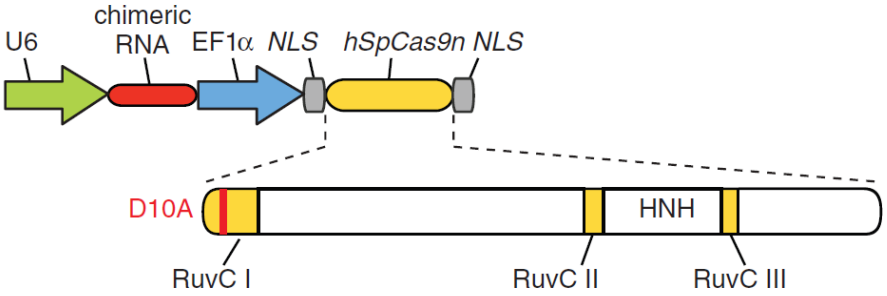

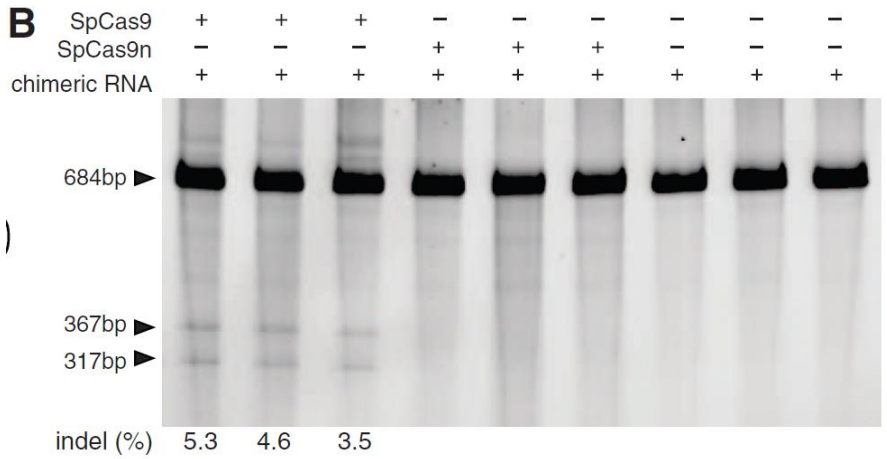

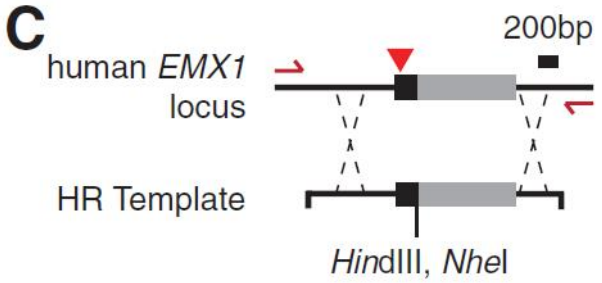

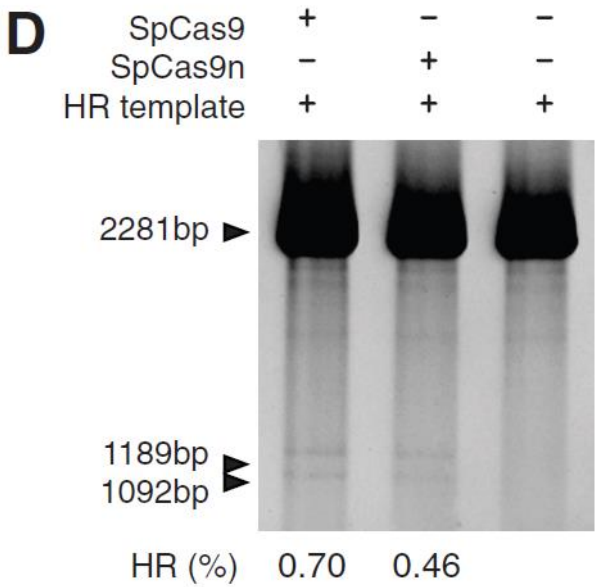

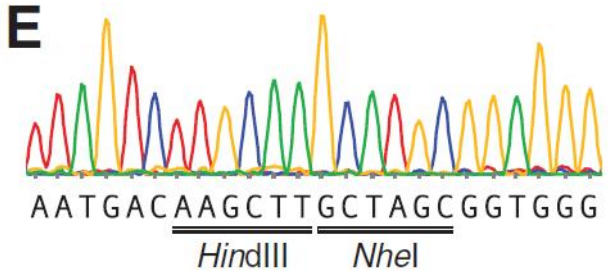

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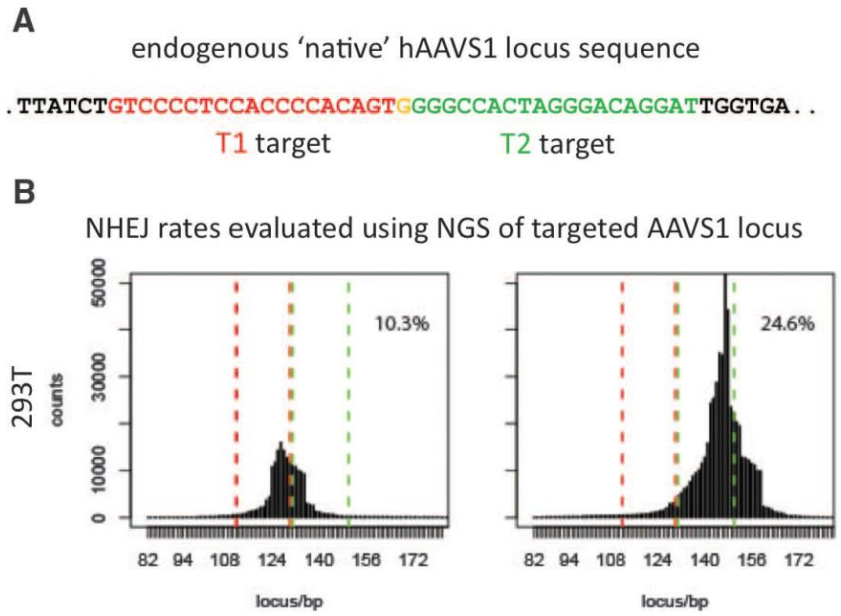
testing the system (NHEJ)

type II CRISPR locus from *S. pvogenes*

- codon-optimized Cas9
- introduced NLS
- transfected HEK293 cells
- MiSeq to analyze deletions
  - gDNA was pooled
  - target region was amplified
  - MiSeq



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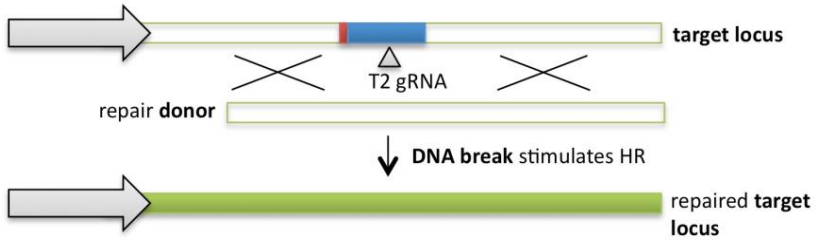
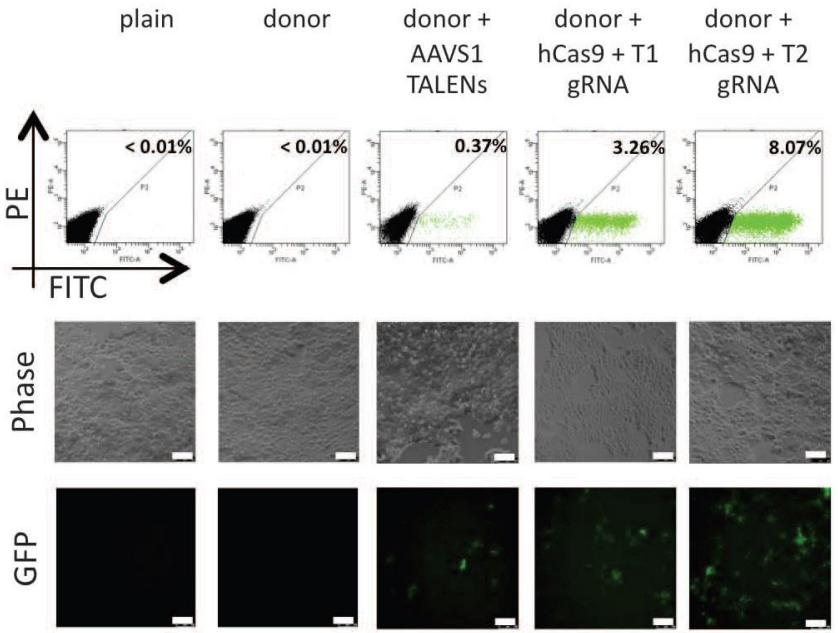
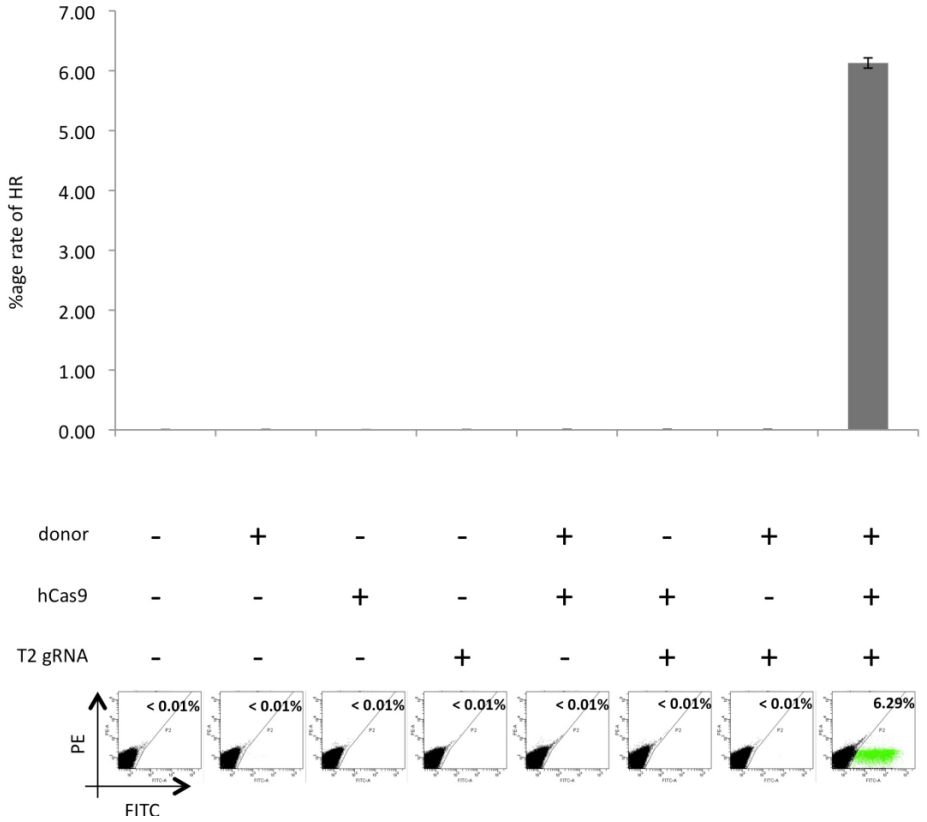
homologous repair/specificity

GFP reporter assay in HEK293T

- stable cell line bearing disrupted GFP
  - insertion of stop
  - a 68 bp from AAVS1 locus
- repair donor to restore GFP expression







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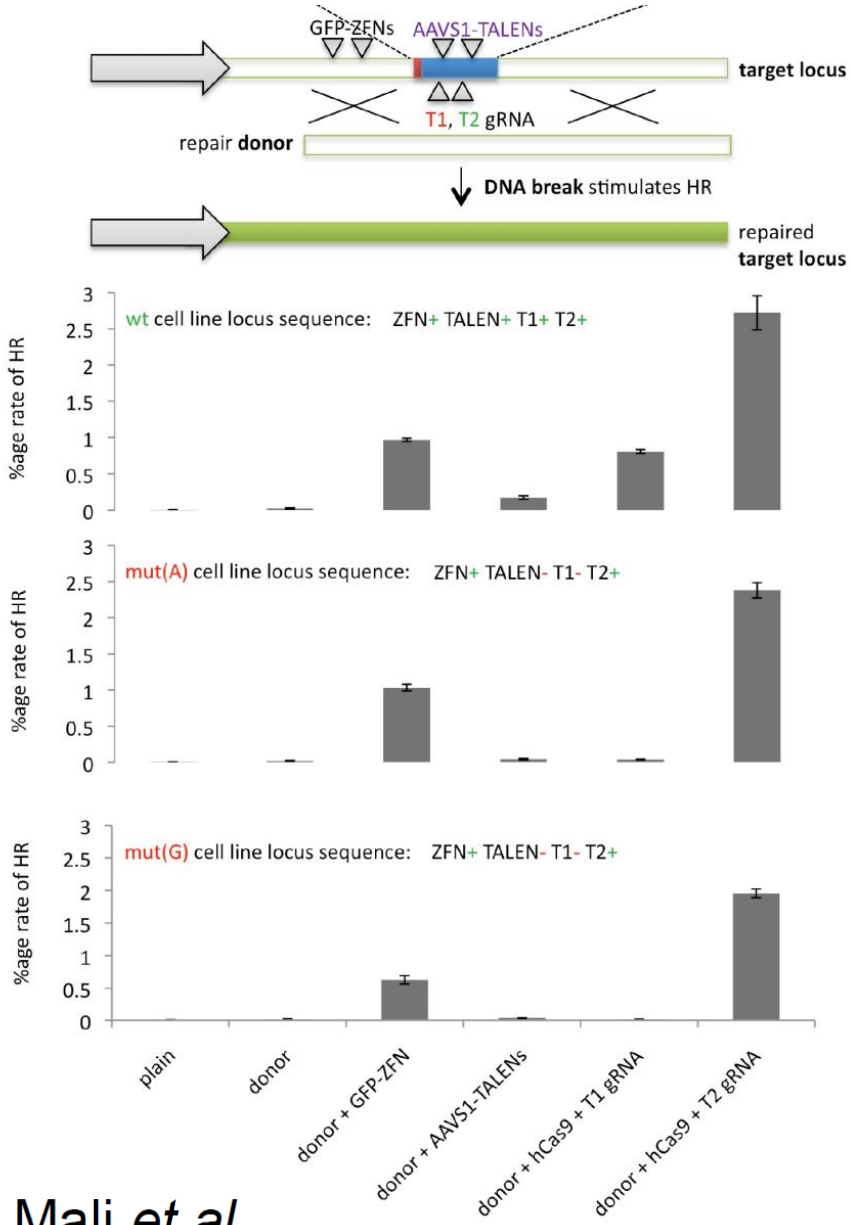

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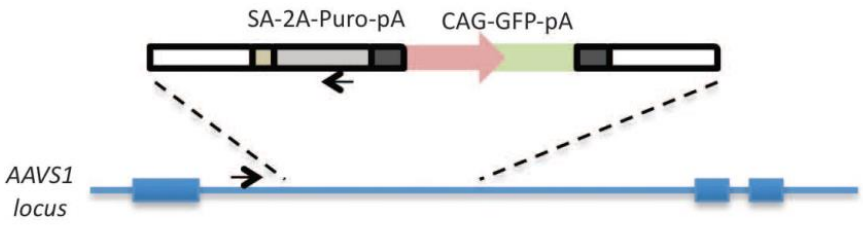
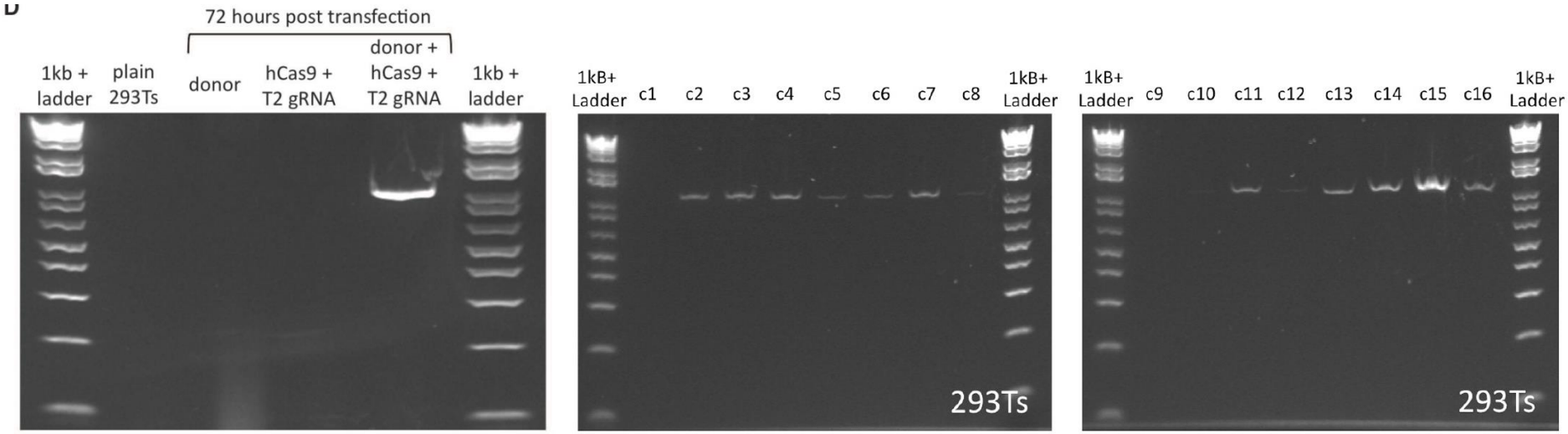
genome editing at endogenous locus



- target endogenous AAVS1 locus
- donor DNA
  - puromycin resistance
  - GFP
- selection of single clones for puromycin



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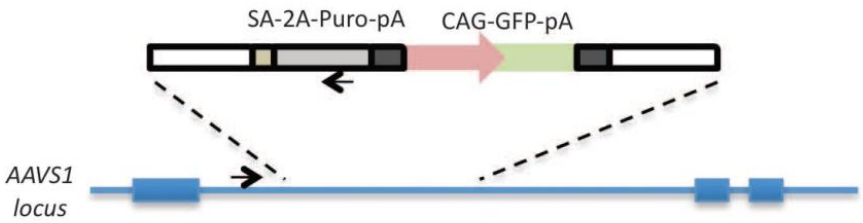

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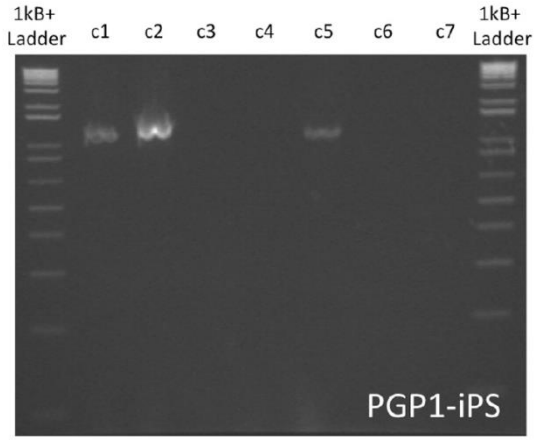

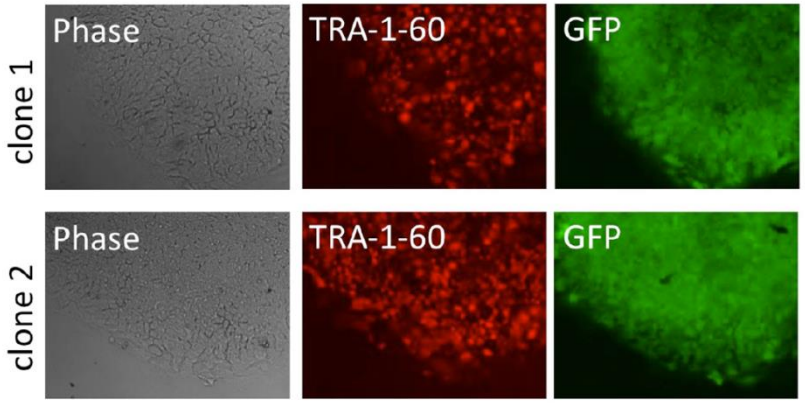

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Thank you very much

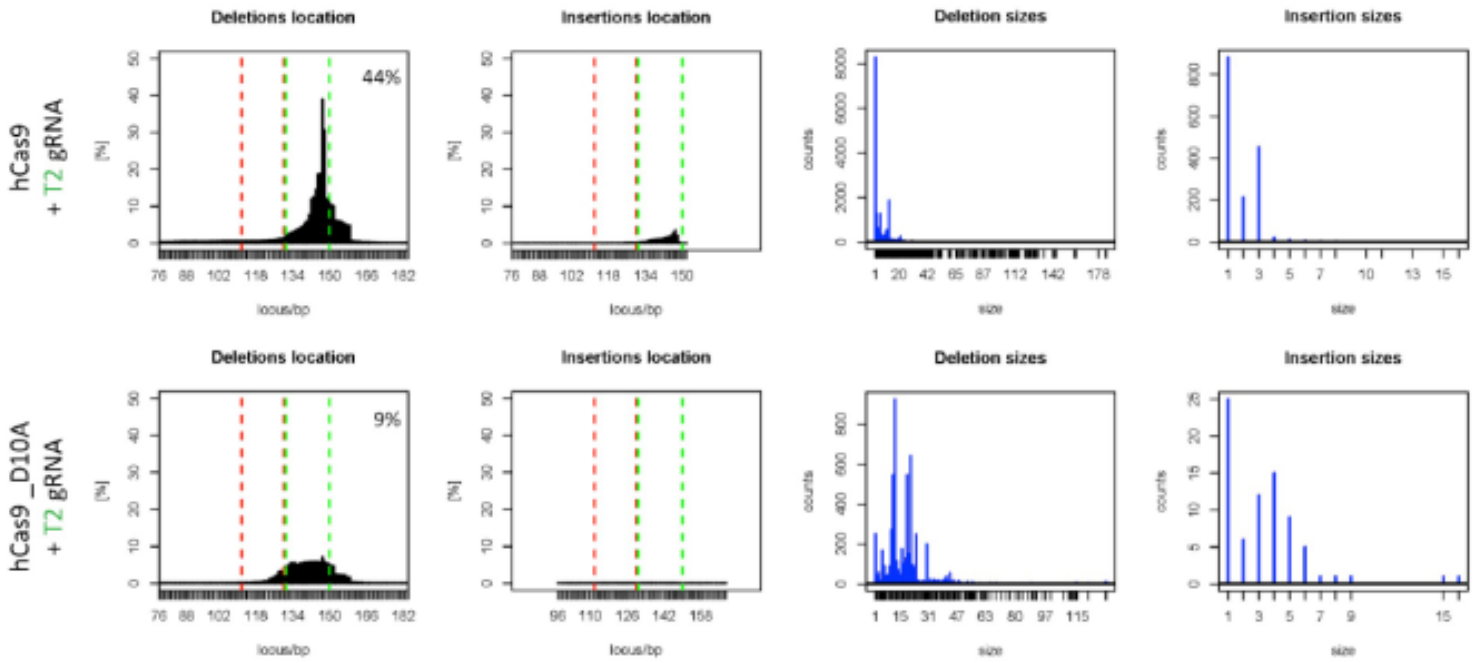
for your attention!!!

D10A mutation

endogenous 'native' hAAVS1 locus sequence
(**293T**)

..TTATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGA..
T2 target

NHEJ rates evaluated using NGS of targeted AAVS1 locus



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